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**Opinion of the Scientific Panel on Animal Health and Welfare on
a request from the Commission related to**

**“Aspects of the biology and welfare of animals used for
experimental and other scientific purposes”**

EFSA-Q-2004-105

Adopted by the AHAW Panel on 14 November 2005

Summary

EFSA was invited by the EU Commission to produce a scientific opinion concerning the “Revision of the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes”.

This scientific opinion was adopted by written procedure on the 14th November 2005, by the Scientific Panel on Animal Health and Welfare (AHAW) after its Plenary Meeting held on the 12th and 13th of October.

According to the mandate of EFSA, ethical, socio-economic, cultural and religious aspects are outside the scope of this opinion.

Summary of the Scientific Opinion for each of the three parts of the Mandate from the Commission:

1. Summary of the need for protection for invertebrates and fetuses and the criteria used (Questions 1 & 2)

The Panel was asked to consider the scientific evidence for the sentience and capacity of all invertebrate species used for experimental purposes and of fetal and embryonic forms to “experience pain, suffering, distress or lasting harm”. Indicators of an animal’s capacity to experience suffering include long-term memory, plasticity of behaviour, complex learning and the possibility of experiencing pain. Some invertebrate species: (i) possess short and long term memory, (ii) exhibit complex learning such as social learning, conditioned suppression, discrimination and generalisation, reversal learning, (iii) show spatial awareness and form cognitive maps, (iv) show deception, (v) perform appropriately in operant studies to gain reinforcement or avoid punishment, (vi) possess receptors sensitive to noxious stimuli connected by nervous pathways to a central nervous system and brain centres, (vii) possess receptors for opioid substances, (viii) modify their responses to stimuli that would be painful for a human after having had analgesics, (ix) respond to stimuli that would be painful for a human in a manner so as to avoid or minimise damage to the body, (x) show an unwillingness to resubmit themselves to a painful procedure indicating that they can learn to associate apparently non-painful with apparently painful events. At a certain stage of development within an egg or the mother, the characteristics listed above may appear. Such information has been used in coming to conclusions about sentience.

Cyclostomes (lampreys and hagfish) have a pain system similar to that of other fish and brains that do not differ much from those of some other fish. There is evidence that cephalopods have adrenal and pain systems, a relatively complex brain similar to many vertebrates, significant cognitive ability including good learning ability and memory retention especially in octopuses, individual temperaments, elaborate signalling and communication systems, especially in cuttlefish and squid that can show rapid emotional colour changes, may live in social groups and have complex social relationships. Nautiloids have many characters similar to those of other cephalopods, they can track other individuals, live for a long time and are active pelagic animals. The largest of decapod crustaceans are complex in behaviour and appear to have some degree of awareness. They have a pain system and considerable

learning ability. As a consequence of this evidence, it is concluded that cyclostomes, all Cephalopoda and decapod crustaceans fall into the same category of animals as those that are at present protected. Using similar arguments, the dramatic evidence of the sensory processing, analytical and prediction ability of salticid spiders provides evidence for awareness greater than in any other invertebrates except cephalopods but we have little evidence of a pain system so do not at present put these spiders in that same category. Free-swimming tunicates are also in this borderline area and social insects and amphioxus are close to it.

Whenever there is a significant risk that a mammalian fetus, or the fetus or embryo of an oviparous animal such as a bird, reptile, amphibian, fish or cephalopod, is for any length of time sufficiently aware that it will suffer or otherwise have poor welfare when a procedure is carried out on it within the uterus or egg, or after removal therefrom, such animals should be included in the list of protected animals. The stage of development at which this risk is sufficient for protection to be necessary is that at which the normal locomotion and sensory functioning of an individual independent of the egg or mother can occur. For air-breathing animals this time will not generally be later than that at which the fetus could survive unassisted outside the uterus or egg. For most vertebrate animals, the stage of development at which there is a risk of poor welfare when a procedure is carried out on them is the beginning of the last third of development within the egg or mother. For a fish, amphibian, cephalopod, or decapod it is when it is capable of feeding independently rather than being dependent on the food supply from the egg.

Precocial oviparous species, some of which are breathing at the time of hatching present much evidence of being aware before hatching and during the last days before hatching,

Even though the mammalian fetus can show physical responses to external stimuli, in some species perhaps for as much as the last third of their development, the weight of present evidence suggests that consciousness is inhibited in the fetus until it starts to breathe air. It is possible that in a mammalian fetus there might be transient episodes of increased oxygenation above the threshold required to support some aspects of consciousness. It is clear that there is a risk, perhaps a small risk, that any mammalian fetus may on occasion be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain. If a mammalian fetus is removed from the mother and starts to breathe, its level of awareness will change to that typical of such animals after parturition. In addition, protection may need to be given against emotional states in pregnant mothers to safeguard subsequent behavioural modification and welfare of the offspring.

When a procedure is performed on a fetus that is likely to produce pain in the newborn or newly-hatched of that species, adequate anaesthesia and analgesia should be given provided that the agents used do not significantly increase the likelihood of fetal mortality. In the circumstance where no suitable anaesthetic or analgesic agents are available, procedures should not be carried out on such fetuses. When the procedure might cause a lasting inflammatory response that persists post-natally, protection should be given against pain and suffering. A schedule of anaesthetics and analgesics that are suitable for use in pregnant animals, and fetuses should be prepared.

2. Summary of the need for purpose breeding of animals and the criteria used (Question 3)

Species listed in Annex I to Directive 86/609/EEC are those that must be 'purpose bred' when used in experiments (unless a specific exemption has been obtained). The criteria for inclusion of species in Annex I have not been clearly defined and hence no information is available on why they were originally included. Therefore, the Commission has asked the EFSA to issue a scientific opinion on the scientific criteria that could be used to determine in which cases animals to be used in experiments should be purpose-bred and, based on these criteria, determine which species currently used in experiments meet these criteria.

It is the opinion of the AHAW panel that including a species as "purpose-bred" within Annex I will confer a considerable degree of assurance that animals of that species will be provided with suitable accommodation, welfare and care practices. As a consequence of health and colony management within breeding establishments, there can be improved confidence in the quality of the animal, resulting in improved science and a reduction in animal numbers required. Taking these factors in isolation, for the great majority of scientific investigations, there would be welfare and scientific merit in recommending that all animals used in scientific procedures be purpose-bred. However, before making such a recommendation, there are a number of other important factors that have to be considered. The consequences of inclusion of all species could, for example, result in loss of genetic diversity, the generation of large numbers of surplus animals and significant delays in scientific progress. A risk assessment approach has therefore been taken to this issue, with the group analysing the potential benefits for and the adverse consequences of the inclusion of each species in Annex I. Two issues have been considered: animal welfare and scientific quality. For each, three steps have been followed: identification of the hazards, exposure assessment and consequence assessment.

The criteria suggested by the Technical Expert Working Group (TEWG) organised by DG ENV (2003) have been considered and incorporated into an assessment process against which the inclusion of each of the commonly used laboratory species was reviewed. The criteria considered by the AHAW panel have been whether legislation already exists to protect animal welfare, genetically altered animals, health and genetic quality of animals, demand, extrapolation of results to farming or to wild populations and capture from the wild.

It is recommended that, wherever possible, animals used should be of a uniform standard so that there is good and effective control over the animals' genetic fidelity, microbial status, nutrition, socialisation to humans and other animals (e.g. ferrets, dogs and even rodents) and environment. Ideally all animals should be purpose bred but, in practice, some exceptions will be necessary. Exceptions should be made to purpose breeding when it is necessary for the research that a particular strain or breed is used, or that scientific progress would be unduly delayed providing that the scientific data resulting from such research were considered likely to be of good quality, i.e. the competent authorities should consider the potential adverse consequences for research should an exemption for the use of non-purpose bred animals be refused (86/609/EEC: Article 19(4)). Genetically altered animals (of all species) should be added to Annex I. The review of all the commonly used laboratory species has concluded that with the exception of quail (*Coturnix coturnix*) all the other species listed should continue to be

purpose-bred and some further species should be added, namely: Chinese hamster (*Cricetus griseus*), Mongolian gerbils (*Meriones unguiculatus*), two *Xenopus* species (*X. laevis* and *X. tropicalis*) and two species of *Rana* (*R. temporaria* and *R. pipiens*).

3. Summary of humane methods of killing animals (Question 4)

Nearly all animals are killed at the end of a research project and it is important that this is done humanely i.e. causing as little suffering as possible for the animals concerned. The majority (85-90%) of animals used in research are small rodents however, of necessity (as we are trying to cover all methods for all animals), much of the Report deals with the methods for large animals. The Opinion of the scientific panel on AHAW is based on the Report annexed to this Opinion that presented recent data building on the three earlier authoritative reports on the humane killing of animals i.e.: 1) the Scientific Report related to welfare aspects of animal stunning and killing methods of the main commercial species of animals (EFSA, 2004, <http://www.efsa.eu.int>); 2) Close *et al.* 1996/1997 (endorsed by the EU for the humane killing of laboratory animals); and 3) the AVMA Report (2000) dealing with methods for all animals. The Opinion does not repeat what is already dealt with in detail in those reports but we have included a section dealing with new data for each method where applicable, and some conclusions and recommendations are retained. The Scientific Report and Opinion deal with the various technical ways of killing animals starting with electrical and mechanical methods, followed by gaseous and then injectable methods. The section on the use of gaseous agents is in some considerable detail as it is the subject of much new data, with more than 20 new papers in the past 10 years, many of them dealing with the commonest laboratory animals. The interpretation of this data has been varied. The recommended methods for each species are given in Tables 1 to 8 at the end of this section but, in general, we have adopted the recommendations given in the existing EU Guidance (Close *et al.*, 1996/97) except where stated. The AHAW panel suggested that these methods could be varied but only with a scientific justification and appropriate authority, i.e. the recommended methods represent the default position. We also address more general issues including ensuring death, training of personnel, killing animals for their tissues and oversight, the choice of method and when this might affect the scientific outcomes, and gathering information on methods used as well as their efficiency and effectiveness.

Key words

Animal research, experimental animals, animal welfare, invertebrate sentience, fetal sentience, purpose breeding, euthanasia.

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1. Terms of Reference

1.1. Background

Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes provides for controls of the use of laboratory animals, it sets minimum standards for housing and care as well as for the training of personnel handling animals and supervising the experiments.

Since 1986, important progress has been made in science and new techniques are now available, such as use of transgenic animals, xenotransplantation and cloning. These require specific attention, which the current Directive does not provide. Nor is the use of animals with a higher degree of neurophysiological sensitivity such as non-human primates specifically regulated. Therefore, Directorate-General Environment (DG ENV) has started revising the Directive.

The revision addresses issues such as compulsory authorisation of all experiments, inspections, severity classification, harm-benefit analysis and compulsory ethical review. Also specific problems relating to the use and acquisition of non-human primates will be tackled.

In 2002, as part of the preparatory work for the revision, DG ENV requested the opinion of the Scientific Committee on Animal Health and Animal Welfare, SCAHAW, on the welfare of non-human primates used in experiments. This Opinion, adopted by SCAHAW on 17 December 2002, was made available to the TEWG. The Opinion already provides some information especially concerning the requirements for purpose-bred animals and the question on gestation for non-human primates.

In 2003, DG ENV organised a Technical Expert Working Group (TEWG) to collect scientific and technical background information for the revision. The experts from Member States, Acceding Countries (which are now the new Member States), industry, science and academia as well as from animal welfare organisations worked through a set of questions prepared by DG ENV. The results of the TEWG provide an important input for the revision of the Directive. However, the TEWG highlighted four specific questions requiring further scientific input. These questions are detailed below. The final reports of the TEWG are provided as background documents.

1.2. Mandate

1.2.1. Question 1 on the sentience of invertebrate species, and fetal and embryonic forms of both vertebrate and invertebrate species

1.2.1.1. Detailed background on invertebrate species

The following definitions are applied in the current Directive:

“animal' unless otherwise qualified, means any live non-human vertebrate, including free-living larval and/or reproducing larval forms...”

“experiment' means any use of an animal for experimental or other scientific purposes which may cause it pain, suffering, distress or lasting harm, including

any course of action intended, or liable, to result in the birth of an animal in any such condition, but excluding the least painful methods accepted in modern practice (*i.e.* 'humane' methods) of killing or marking an animal”

The TEWGs and other experts recommended to enlarge the scope to include **invertebrate species** provided there is sufficient scientific evidence as to their sentience and capacity to “experience pain, suffering, distress or lasting harm”. Certain species of invertebrates are already included in the national legislation of some countries, both within and outside the EU (*e.g.* UK, some Scandinavian countries, Australia Capital Territories, New Zealand). The UK currently only includes *Octopus vulgaris* in its national legislation but is considering the inclusion of additional cephalopod species.

1.2.1.2. Terms of reference of question 1

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- the sentience and capacity to “experience pain, suffering, distress or lasting harm” of all invertebrate species used for experimental purposes.

1.2.2. ***Question 2 on fetal and embryonic forms***

1.2.2.1. Detailed background on fetal and embryonic forms

The definition of ‘animal’ in the current Directive excludes fetal or embryonic forms.

According to TEWG and other experts, fetal and embryonic forms should be brought under the scope of the Directive in case there is enough scientific evidence on their capacity to “experience pain, distress or lasting harm”.

Some Member States have included in their national legislation such forms beyond a certain stage of pregnancy. A criterion for determining the appropriate stage of pregnancy may be the development of the cerebral cortex and when it reaches a stage at which it can register sensory experiences.

The view of several members of the TEWG was that a time limit of half way through the gestation period should be used, at least for all large mammalian species other than rodents. This was based on data relating to sheep and non-human primates whilst providing for a ‘safety margin’ with regard to the ability of fetuses/embryos of these species to feel pain. However, the TEWG could not reach a consensus on when a rodent fetus or new-born may be capable of suffering, although they suggested that the final 20% of pregnancy may be appropriate for rodent and poultry species.

1.2.2.2. Terms of reference of question 2

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- The stage of gestation after which the fetus/embryo of the species in question is assumed to be capable of “experiencing pain, suffering, distress or lasting harm”,
- whether a generic rule for a cut-off point for the advancement of gestation can be indicated for those species where insufficient scientific data exist to establish a species-specific cut-off point.

1.2.3. Question 3 on purpose-bred animals

1.2.3.1. Detailed background on purpose-bred animals

Species listed in Annex I to Directive 86/609/EEC are those that must be ‘purpose bred’ when used in experiments (unless a specific exemption has been obtained). The criteria for inclusion of species in Annex I have not been clearly defined and no information is available on why the various species were originally included.

For example, mini-pigs which have become a widely-used laboratory species, obtained from commercial suppliers where they are bred in a controlled environment similar to that to be experienced at user facilities. According to the TEWG, their inclusion in Annex I would therefore appear logical and in the interest of sound principles of scientific research and welfare. Other species to be considered for inclusion could be ferrets and some hamster species in addition to *Mesocricetus auratus*. Conversely, the current inclusion of quail (*Coturnix coturnix*) should be re-considered.

The TEWG proposed multiple criteria as a basis for species inclusion into Annex I, such as:

- numbers of animals required for procedures;
- the type of procedures (*e.g.* farm animal studies/population studies);
- animal welfare aspects;
- practical and commercial aspects of establishing breeding;
- disease-free requirements;
- specific animal welfare aspects such as social deprivation, confinement and other aspects of sudden involuntary changes of living environment (use of pet or stray animals as experimental animals.)

1.2.3.2. Terms of reference of question 3

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- the scientific criteria that could be used to determine in which cases animals to be used in experiments should be purpose-bred, in order to safeguard *inter alia* animal welfare, using the proposal of the TEWG. The proposed criteria should also take into account other factors such as current and future needs, practicability or any specific scientific requirements.

- Based on these criteria, determine which species currently used in experiments meet these criteria.

1.2.4. Question 4 on humane methods of euthanasia

1.2.4.1. Detailed background on humane methods of euthanasia

Some experimental animals are only bred to be euthanised for the purpose of using their tissues and/or organs, e.g. in the development and application of *in vitro* methods. To ensure highest possible animal welfare standards in the EU, it needs to be defined which methods of killing are scientifically the most humane and appropriate for different species of experimental animals.

1.2.4.2. Terms of reference of question 4

In view of the above, the Commission asks the European Food Safety Authority to issue a scientific opinion on:

- the methods of euthanasia which could, on the basis of current scientific knowledge and respecting good animal welfare, be justified as being the most appropriate per type of species.
- To specify these methods and their suitability for different species most commonly used in experiments.

1.3. Approach

This Scientific opinion is a scientific assessment of the needs for a revision of the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. It has been based on the Scientific Report accepted by the EFSA AHAW Panel. In drafting this Scientific Opinion, the panel did not take into consideration any ethical, socio-economic, human safety, cultural or religious aspect of the topic, the emphasis has been to look at the scientific evidence and to interpret that in the light of the terms of reference.

The three working groups (WGs) were set up to address these questions with relevant experts being appointed as members.

This scientific opinion comprises 3 parts / Chapters in response to the 4 questions posed by the Commission (see Section 1.2). Questions 1 and 2 overlapped in scope essentially dealing with sentience of both fetal forms and invertebrates, and are addressed in *Chapter 2*. Questions 3 and 4 remain separate and as they are given in the mandate. They cover purpose breeding of animals (*Chapter 3*), and euthanasia of the commonly used species (*Chapter 4*). It was decided that if in Chapter 2, some species were to be recommended to receive protection, then the report and opinion should also address the question of whether they should be purpose bred in Chapter 3, and how they could be humanely killed in Chapter 4.

A full assessment and the risk profiles can be found in the Scientific Report, published on the EFSA web site, which were drafted by three Working Groups set up by the AHAW Panel.

The Tables 1-8, at the end of the Opinion are taken from Close *et al.* 1996, 1997 and have been modified according to the Scientific Report and update the EU recommendations on humane methods of killing protected animals.

As part of the approach by EFSA two Stakeholders consultation meetings were held on 18th February and the 31st August 2005. At the first meeting Stakeholders were asked to comment on the mandate from the Commission and on the proposed method working. Stakeholders were asked to propose scientific experts, not organisational representatives, that EFSA could call on for help in the working groups (WGs), and to provide any background scientific papers that the WGs might find useful. The suggestions made were very helpful. The scientific experts were selected by EFSA on the basis that they had made a significant contribution to the topic under review in the past 5 years or, where there was no or little scientific data, that they had relevant and appropriate experience. A draft of the Scientific Report (including the proposed recommendations) was sent out on the 28th July for the Stakeholders to seek comments from their members in time for the meeting on the 31st August. At that meeting views were sought from the Stakeholders on the draft Report and the WG's conclusions and recommendations. After Aug 31st Stakeholders were given another 7 days to reconsider their views in the light of the responses from other Stakeholders to make a written response to EFSA on their final views. These views were then considered by the WGs in their preparation of their final Report.

2. QUESTION ON THE SENTIENCE OF INVERTEBRATE SPECIES, AND ON FETAL AND EMBRYONIC FORMS OF BOTH VERTEBRATE AND INVERTEBRATE SPECIES.

All invertebrate animals were considered and our recommendations propose some groups as “protected animals”.

2.1. Memory and Learning in Invertebrates

Conclusion: The memory and learning of invertebrates has been widely investigated. It has been shown that invertebrates are capable of learning in several ways very similar to vertebrates: for example, slugs are capable of first- and second-order conditioning, blocking, one-trial associative learning and appetitive learning (Yamada *et al.*, 1992). In a comprehensive review of invertebrate learning and memory, Carew and Sahley (1986, p. 473) were so impressed by the learning capabilities of invertebrates they were moved to write -

"In fact, the higher-order features of learning seen in some invertebrates (notably bees and *Limax*) rivals that commonly observed in such star performers in the vertebrate laboratory as pigeons, rats, and rabbits."

2.2. Nociception and Pain in Invertebrates

Summary: In respect to brain and nervous complexity, there is no doubt that invertebrates have simpler nervous systems than vertebrates, but does this mean they are unable to suffer? The cerebral cortex is thought to be the seat of consciousness in humans (Smith and Boyd 1991). In fact, pain and suffering are sometimes defined in terms of neural activity in the cerebrum, which makes it a rather circular argument to then dismiss the possibility of invertebrates being capable of suffering because they lack such a structure. It is possible that other structures, as yet undetermined, within the brain or elsewhere fulfil a similar function to the cerebrum in terms of processing information related to suffering. Analogous yet disparate structures have evolved throughout the animal kingdom. For example, the compound eye of some invertebrates is strikingly different in form from the mammalian eye, yet they both achieve the same function - they allow the animal to perceive light. Parts of the nervous system of invertebrates that are not the anterior brain are capable of controlling breathing, movement and learning (e.g. octopuses, cockroaches). Possibly, areas of invertebrate nervous tissue have evolved abilities analogous to the cerebrum of mammals and give these animals the capacity to suffer. Above all, we should remember that absence of evidence is not evidence of absence.

Conclusion 1: It is often suggested that indicators of an animal’s capacity to experience suffering include long-term memory, plasticity of behaviour, and ‘higher’ learning. Many invertebrate species:

- Possess short and long term memory;
- Exhibit higher learning such as social learning, conditioned suppression, discrimination and generalisation, reversal learning;

- Show great spatial awareness and form cognitive maps (possibly indicating self-awareness);
- Appear to show deception (possibly indicating they possess a theory of mind);
- Perform appropriately in operant studies to operate a manipulandum or change the environment in some way to gain reinforcement or avoid punishment.

Conclusion 2: Regarding the possibility of invertebrates experiencing pain, many invertebrate species:

- possess receptors sensitive to noxious stimuli connected by nervous pathways to a central nervous system;
- possess brain centres;
- possess nervous pathways connecting the nociceptive system to the brain centres;
- possess receptors for opioid substances;
- after having had analgesics, modify their responses to stimuli that would be painful for a human;
- respond to stimuli that would be painful for a human in a functionally similar manner (that is, respond so as to avoid or minimise damage to the body);
- show behavioural responses that persist and show an unwillingness to resubmit to a painful procedure; they can learn to associate apparently non-painful with apparently painful events.

2.3. Non-vertebrate groups

2.3.1. *Cyclostomes (lampreys and hagfish).*

Conclusion: Cyclostomes have a pain system similar to that of other fish and brains which do not differ much from those of some other fish.

Recommendation: Cyclostomes should be in Category 1 (see Section 2.5) and so receive protection.

2.3.2. *Amphioxus*

Conclusion: In general, insufficient is known about whether amphioxus are able to experience pain and distress

Recommendation: Given our present state of knowledge amphioxus should be in Category 3 (see Section 2.5) and not receive protection at present.

2.3.3. *Tunicate*

Conclusion: Free swimming larval forms and pelagic adult tunicates show responses which may indicate complex processing of stimuli but little information on this topic

is available. The free-swimming adult and larval tunicates are similar in form and in some aspects of behaviour to amphibian tadpoles but most are smaller.

Recommendation: Given our present state of knowledge tunicates should be in Category 3 (see Section 2.5) and not receive protection at present.

2.3.4. Hemichordata such as *Balanoglossus*

Conclusion: *Balanoglossus*, the acorn worm, lives on the bottom in marine environments. There is no indication from its behaviour that it has any sophisticated brain function.

Recommendation: Given our present state of knowledge *Balanoglossus* should be in Category 2 (see Section 2.5) and not receive protection.

2.3.5. Cephalopods (octopods, squid, cuttlefish, nautiloids)

Conclusion: There is evidence that cephalopods have a nervous system and relatively complex brain similar to many vertebrates, and sufficient in structure and functioning for them to experience pain. Notably, they release adrenal hormones in response to situations that would elicit pain and distress in humans, they can experience and learn to avoid pain and distress such as avoiding electric shocks, they have nociceptors in their skin, they have significant cognitive ability including good learning ability and memory retention, and they display individual temperaments since some individuals can be consistently inclined towards avoidance rather than active involvement. Most work on learning ability has been carried out in the non-social but visually very competent *Octopus vulgaris*. All squid, cuttlefish and octopods (coleoid cephalopods) studied have a similar ability to sense and learn to avoid painful stimuli, and many are more complex and more likely to experience pain and distress than *O. vulgaris*. Learning is involved in most signalling and the most elaborate signalling and communication systems occur in cuttlefish and squid that can show rapid emotional colour changes and responses to these. Indeed many of these animals live in social groups and hence may have levels of cognitive ability like those of vertebrates that have complex social relationships. Nautiloids have less complex behaviour than coleoid cephalopods and much less is known about their learning ability. They use odour discrimination to find mates and respond to and track other individuals of their own species (Basil 2001, 2002) but little is known about their pain system and it is not clear whether they are as capable of suffering as other cephalopods. However, they live for a long time and are active pelagic animals so we cannot be sure about their level of awareness.

Recommendation: All cephalopods should be in Category 1 (see Section 2.5) and so receive protection.

2.3.6. Land gastropods

Conclusion: Snails and slugs can show quite complex learning but the relatively slow locomotion of most of them does not enable them to show rapid escape responses, except for localised movements like eye withdrawal. The case for a substantial degree of awareness would appear to be weak.

Recommendation: Given our present state of knowledge land gastropods should be in Category 2 (see Section 2.5) and not receive protection

2.3.7. Tectibranch and nudibranch molluscs

Conclusion: The most active marine gastropod molluscs are the tectibranchs, such as *Aplysia* and some of the nudibranchs (sea slugs). Much research has been carried out on the nervous system of *Aplysia* and its relatives. Evidence of learning and flexibility of behaviour is considerable but there are also studies showing very rigid responses. Nudibranchs appear to be less flexible than some tectibranchs.

Recommendation: Given our present state of knowledge tectibranch and nudibranch molluscs should be in Category 2 (see Section 2.5) and not receive protection.

2.3.8. Social insects

Conclusion: The social ants and bees, and to a lesser extent the wasps and termites, show considerable learning ability and complex social behaviour. There is evidence of inflexibility in their behaviour but the trend in recent research has been to find more flexibility. The small size of the brain does not mean poor function as the nerve cells are very small. A case might be made for some bees and ants to be as complex as much larger animals. They might be aware to some extent but we have little evidence of a pain system.

Recommendation: Given our present state of knowledge social insects should be in Category 3 (see Section 2.5) and not receive protection

2.3.9. Other insects

Conclusion: There is a difference in complexity of behaviour between the social and non-social insects. However, learning is clearly possible in these animals. There is little evidence of awareness but few people have looked for it.

Recommendation: Given our present state of knowledge other insects should be in Category 2 (see Section 2.5) and not receive protection.

2.3.10. Spiders, especially jumping spiders

Conclusion: In recent years, dramatic evidence has been produced of the sensory processing, analytical and prediction ability of salticid spiders. The eyes are large and complex and although the brain is composed of a relatively small number of cells, the level of processing is considerable and sophisticated, if rather slow. Evidence for awareness is greater than in any other invertebrates except cephalopods but we have little evidence of a pain system.

Recommendation: Given our present state of knowledge spiders should be in Category 3 (see Section 2.5) and not receive protection at present.

2.3.11. Decapod crustaceans (lobsters, crabs, prawns etc.)

Conclusion: The largest of these animals are complex in behaviour and appear to have some degree of awareness. They have a pain system and considerable learning

ability. Little evidence is available for many decapods, especially small species. However, where sub-groups of the decapods, such as the prawns, have large species which have been studied in detail they seem to have a similar level of complexity to those described for crabs and lobsters.

Recommendation: All decapods should be in Category 1 (see Section 2.5) and so receive protection.

2.3.12. Isopods (woodlice and marine species)

Conclusion: Learning is clearly possible in these animals and some of them live socially. The degree of complexity of functioning is lower than that of the larger decapods or many insects and spiders.

Recommendation: Given our present state of knowledge isopods should be in Category 2 (see Section 2.5) and not receive protection.

2.3.13. Other phyla (e.g. Annelida, Echinodermata, Platyhelminthes, and Nematoda) not described above, as well as other Classes, have been considered but are not thought to need protection and therefore have all been placed in Category 2

2.4. Fetal and embryonic animals which might be protected

Summary: Even though the mammalian fetus can show physical responses to external stimuli, the weight of present evidence suggests that consciousness does not occur in the fetus until it is delivered and starts to breathe air. However, events *in utero* can influence the behaviour of the individual once it is born, and some of those effects could be important to its subsequent welfare. Precocial oviparous species present much evidence of being conscious at hatching, and during the last days before hatching.

Conclusions

1. Precocial oviparous species, some of which are breathing at the time of hatching present much evidence of being aware before hatching and during the last days before hatching, perhaps for as much as the last third of their development. They are often capable of independent life if removed from the egg during the last few days before hatching. Altricial oviparous species and species with larval forms do not develop awareness until a later age. For all oviparous species and especially for the many precocial species there is a high risk that fetuses in the egg during the last part of incubation will be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain.
2. Even though the mammalian fetus can show physical responses to external stimuli, the weight of present evidence suggests that consciousness is not the normal state in the fetus until it is delivered and starts to breathe air.
3. It is possible that in a mammalian fetus there might be transient episodes of increased oxygenation above the threshold required to support some aspects of consciousness. We have insufficient knowledge to conclude whether or not this occurs in all, or even any, fetuses. It is clear that there is a risk, perhaps a small risk, that any mammalian

fetus may on occasion be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain.

4. If a mammalian fetus is removed from the mother and starts to breathe, its level of awareness will change to that typical of such animals after parturition.
5. Emotional stresses experienced by a pregnant mother mammal can influence the behaviour of the offspring after it is born and some of those effects could be important to the offspring's subsequent welfare. It may be that the effects are mediated via nutrition or other means from the mother or it may be that the fetus experiences these effects directly.
6. The fetus in oviparous species, especially those which are precocial, can react to and learn from experiences received during the last few days of incubation.
7. For most vertebrate animals and cephalopods, the stage of development at which there is little risk of poor welfare when a procedure is carried out on them is the beginning of the last third of development during incubation or pregnancy. Before that time the risk to animal welfare is not thought to be significant. For some species this may be earlier but we have not been able to compile a database of species and fetal forms at which some form of protection was assessed as being necessary.
8. For fish, amphibians and cephalopods which develop in water, functioning has many similarities to that of adult fish once they start to feed independently rather than being dependent on the food supply from the egg.
9. The protection of the animals recommended to be included as a protected animal in Chapter 2 poses practical problems during the early stages of their development when they will be microscopic.

Recommendations

1. Whenever there is a significant risk that a mammalian fetus or the fetus or embryo of an oviparous animal such as a bird, reptile, amphibian, fish or cephalopod is for any length of time sufficiently aware that it will suffer or otherwise have poor welfare when a procedure is carried out on it within the uterus or egg, such animals should receive protection. The stage of development at which this risk is sufficient for protection to be necessary is that at which the normal locomotion and sensory functioning of an individual independent of the egg or mother can occur. For air-breathing animals this time will not generally be later than that at which the fetus could survive unassisted outside the uterus or egg.
2. Once a fetus is removed from the uterus or egg, if it is capable of breathing such animals should receive protection.
3. As a guideline, and because of the risk that even mammals in utero may sometimes be aware at times before parturition, when a procedure is performed on a fetus that is likely to produce pain in the newborn of that species, adequate anaesthesia and analgesia should be given provided that the agents used do not significantly increase the likelihood of fetal mortality. In the circumstance where no suitable anaesthetic or analgesic agents are available, procedures should not be carried out on such fetuses.

When the procedure might cause a lasting inflammatory response that persists post-natally, protection should be given against pain and suffering.

4. A schedule of anaesthetics and analgesics that are suitable for use in pregnant animals, oxygenated fetuses and newborn animals should be prepared.
5. Protection against pain and distress during any procedures that might cause these, should be given to any precocial birds or reptiles, for example domestic chicks, that are breathing before hatching.
6. In order to avoid the risk that a fetus, whether it is developing in the mother or in an egg outside the mother, will be affected by some experimental procedures in such a way that its welfare is poor, sometimes because it is suffering pain, it should receive protection if it is in the last third of its development during incubation or pregnancy. This recommendation should be taken together with those above in order that any species at an appropriate stage of development will be protected.
7. Protection may need to be given against emotional states in pregnant mothers to safeguard subsequent behavioural modification and welfare of the offspring. This needs to be considered on a case-by-case basis.
8. In order to avoid the risk that a fish, amphibians, cephalopods or decapods will be affected by some experimental procedures in such a way that its welfare is poor, sometimes because it is suffering pain, it should be included in the list of protected animals receive protection if it is capable of feeding independently rather than being dependent on the food supply from the egg. This food supply is carried around by young fish etc. after emerging from the egg but the young animal is not independent of it for some time. The point of development at which complex function is possible is predicted well by independent feeding.

2.5. Implications for the definition of a “protected animal”

While the principal reason for the existence of legislation is to harmonise the implementation of the Three Rs of Replacement, Reduction and Refinement. This would imply that it is important to define the term “protected animal” and other animal forms which are to be protected during experimental and other research work.

When experiments are carried out *in vivo* (literally meaning scientific procedures involving a living animal with its whole body systems intact) a key point is whether the animal is able to experience pain and distress and other forms of suffering. The inclusion, therefore, of invertebrates and fetal forms from certain stages of gestation, as well as vertebrates, based on the information given in Chapter 2, is essential information for risk management. The WG have tried to give guidance on that issue with the criteria used to do so. The use of terms such as free-living, capable of independent feeding etc are fraught with difficulties as they do not allow all animal forms at all stages of development to be clearly distinguished on the basis of their ability to experience pain, distress etc. There are however, some worthwhile analogies that can be made, so that more complex forms are more likely to be sentient than simple forms i.e. independent feeders are more likely to be sentient than sessile free living forms,

The WG is proposing therefore, that three categories be established.

Category 1 - The scientific evidence clearly indicates that those groups of animals are able to experience pain and distress, or the evidence, either directly or by analogy with animals in the same taxonomic group(s), are able to experience pain and distress.

Category 2 - The scientific evidence clearly indicates that those groups of animals are NOT able to experience pain and distress, or the evidence, either directly or by analogy with animals in the same taxonomic group(s), are unable to experience pain and distress.

Category 3 - Some scientific evidence exists that those groups of animals are able to experience pain and distress, either directly or by analogy with animals in the same taxonomic group(s), but it is not enough to make a reasonable risk assessment on their sentience to place them in either Category 1 or 2.

Any such categorisation of animals and their forms will need updating as scientific knowledge accumulates.

3. QUESTION ON PURPOSE-BRED ANIMALS

Including a species as "purpose-bred" within Annex I will confer a considerable degree of assurance that animals of that species will be provided with suitable accommodation, welfare and care practices. As a consequence of health and colony management within breeding establishments, there can be improved confidence in the quality of the animal, resulting in improved science and a reduction in animal numbers required. Taking these factors in isolation, for the great majority of scientific investigations, there would be welfare and scientific merit in recommending that all animals used in scientific procedures be purpose-bred. Before making such a recommendation, there are a number of other important factors that have to be considered and there will have to be exceptions to this in some areas of research e.g. studies into the normal biology of a species, commercial strains and veterinary clinical research. The consequences of inclusion of all species could, for example, result in loss of genetic diversity, the generation of large numbers of surplus animals and significant delays in scientific progress, breeding wild animals in captivity could be detrimental to their health and welfare.

A risk assessment approach has therefore been taken to this issue, with the group analysing the potential benefits and adverse consequences of inclusion of each species in Annex I.

3.1. Key criteria to be considered for being purpose bred and inclusion in Annex I:

1. Other legislation already protecting animal welfare - Absence of any relevant animal welfare legislation is a reasonable criterion for inclusion into Annex I.
2. Genetically altered animals - Welfare requirements for GAA are more likely to be met if purpose bred.
3. Health and genetic fidelity of animals - Animals that are purpose bred are likely to be of high health status and genetic fidelity.
4. Demand - Species with low or widely fluctuating demands are reasons for not including in the Annex I.
5. Extrapolation of results to farming or to wild populations - Species primarily used in studies where the data are extrapolated, for example, to commercial farming production, or ecological studies in wild animals, is a reason for not including them in Annex I.
6. Capture from the wild - Capturing a species from the wild for use in a laboratory is a major welfare concern and is, therefore, an important criterion for inclusion of the species in Annex I. Purpose breeding primates may in some cases be the only alternative source to capture in the wild.

3.2. Conclusions and Recommendations

Specific conclusions and recommendations with regard to species where changes might be made to their particular purpose bred status are given in the Tables from the Scientific Report (Appendices 1 - 7). See below.

Conclusion 1: Purpose-breeding is considered to be an important measure of producing high quality animals for research, to minimise inter-animal variability thus reducing the overall number required, and to promote improved welfare for the animals as well as the scientific outcomes. Therefore, the most appropriate animals in most cases will be purpose bred.

Recommendation 1: For most areas of research it is appropriate that the animals used should be of a uniform standard so that there is good and effective controls over the animals' genetic fidelity, microbial status, nutrition, socialisation to humans and other animals (e.g. ferrets, dogs and even rodents) and environment. The most appropriate animals should be used for research. In most cases, these will be purpose bred. The use of non-purpose breed animals will require appropriate justification.

Conclusion 2: Purpose breeding some species of animals that are not frequently used, or that are needed for a narrow area of research, or whose demand fluctuates widely, or that are protected by other legislation, or that have long gestation periods, could all result in difficulties in obtaining suitable animals for research programmes. At best this could delay scientific progress and could result in the abandonment of some research programmes.

Recommendation 2: Exceptions should be made to purpose breeding when it is necessary for the research that a particular strain or breed is used, or that scientific progress would be unduly delayed providing that the scientific data resulting from such research was of good quality, i.e. the competent authorities should consider the potential adverse consequences for research should an exemption for the use of non-purpose bred animals be refused (Council Directive 86/609/EEC: Article 19(4)).

Conclusion 3: Welfare requirements for genetically altered animals are more likely to be met if they are purpose bred.

Recommendation 3: Genetically altered animals should be purpose-bred unless an exemption is authorised by the Competent Authority. An exemption should only be approved where good evidence is provided that any genetic alteration does not cause the animals pain, suffering, distress or lasting harm, and is unlikely to cause such suffering in subsequent generations.

Conclusion 4: The process of genetic alteration can produce, either intentional adverse effects, or as an unexpected consequence of the alteration produce unexpected adverse effects, both of which require that animals are provided with specialist husbandry and care. Failure to provide appropriate accommodation and care practices could adversely affect animal welfare and scientific outcomes.

Recommendation 4: Genetically altered animals of all protected species and forms should be added to Annex I but can be exempted if it is shown that there are, or likely to be, no serious adverse effects on the animals in their future environment and the way they are used (e.g. future breeding programmes).

Conclusion 5: Because the welfare of the animals and the scientific validity of the data are inextricably linked with good quality care and husbandry of animals it is important that all those who come into contact with the animals are adequately educated, trained and skilled on an ongoing basis. This is more likely to happen when animals are purpose bred.

Recommendation 5: In registered breeding and supplying establishments personnel should be properly trained and only competent staff should be given responsibility for the care and husbandry of animals.

Conclusion 6: Inclusion of a species in Annex I requires that animals will be purpose-bred for research purposes. The inclusion of such an Annex is considered to have welfare and scientific benefits. The review of all the commonly used laboratory species has concluded that with the exception of quail (*Coturnix coturnix*) all the other species listed should continue to be purpose-bred. The review also concluded that some further species should be added.

Recommendation 6: The criteria for purpose bred animals and the current guidelines on accommodation and care included in Annex II (and any revision) which is expected in the future to be revised to reflect the revised Appendix A of Council of Europe Convention (1986) ETS 123 should apply irrespective of the origin of the experimental animals. In making this recommendation it is appreciated that in practice not all establishments will at present meet these criteria, but nonetheless all establishments should be strongly encouraged to make progress towards these in a timely manner.

Conclusions in relation to specific species used in research

Hamsters

Conclusion 7: Syrian hamsters are the most commonly used of all the ‘hamster types’ and, at present, are included in Annex I. However, from an analysis of scientific papers through PUBMED, Chinese hamsters are also commonly used, and only very few European and Djungarian hamsters.

Arguments against inclusion of all hamster species: The small numbers of European and Djungarian hamsters used would make difficulties to match supply and demand leading to delays in scientific programmes

Arguments for inclusion of all hamster species: It would be likely that there would be an improved and more uniform health quality. Moreover no other welfare legislation exists.

Recommendation 7: Retain Syrian hamsters and include Chinese hamsters. No compelling need to include any other hamster species.

Gerbils

Conclusion 8: The commonest gerbil used in research is the Mongolian (*Meriones unguiculatus*) which is not in Annex I.

Arguments against inclusion: Difficulties to match supply and demand that may lead to some delays in scientific programmes;

Arguments for inclusion: Better and more uniform health quality; improved accommodation leading to reduced behavioural abnormalities; no other suitable welfare legislation

Recommendation 8: To include Mongolian gerbils in Annex I (*Meriones unguiculatus*).

Quail

Conclusion 9:

Arguments for inclusion: There may possibly be better protection for quail if listed in Annex I, through improved accommodation and care practices.

Arguments against inclusion: Small numbers of *Coturnix coturnix* used. Few breeding establishments – difficult to match supply and demand.

Recommendation 9: There is no compelling need to retain *Coturnix coturnix*, nor to include any other species of quail.

***Xenopus* species (*laevis* and *tropicalis*), *Rana* species (*temporaria* and *pipiens*)**

Conclusion 10:

Arguments against inclusion: Wide range of species but for many species only small numbers are used. Production of the less commonly used species, e.g. newts, salamanders (including axolotls) may not be practicably viable due to the very small numbers used. The purpose breeding of *Xenopus laevis* and *tropicalis* may prove to have economies of scale that make it viable. Potentially high cull rates, difficulties to match supply and demand leading to delays in scientific programmes, lack of information on husbandry and care practices.

Arguments for inclusion: better and more uniform health quality, increasing numbers of some species, no other welfare legislation, elimination of zoonotic diseases, no animals taken from wild.

Recommendation 10: *Xenopus* species (*laevis* and *tropicalis*) and *Rana* (*Rana temporaria* and *R. pipiens*) should be purpose bred.

Invertebrates such as cephalopods, cyclostomes, decapods.

Conclusion 11: The recommendation from Chapter 2 is for these phyla to receive protection during experimental work due to their potential to experience pain and distress.

Recommendation 11: If the recommendations put forward in Chapter 2 are accepted, there is no compelling need to include any of these invertebrate species, at the moment, in those to be purpose bred.

4. QUESTION ON HUMANE METHODS OF EUTHANASIA

4.1. Reasons for euthanasia:

The reasons for killing animals have also to be considered, as some methods may cause more pain and distress than others. For example, breeding more animals than are required simply to have them available on demand, and then killing those that have not been used. This is especially true for animals that have a painful harmful defect caused for example by a genetic alteration. Sometimes killing of surplus is inevitable as in the breeding of some transgenic or mutant animals as only a particular genotype is wanted, and uses cannot be found for the surplus animals. On other occasions, breeding strategies can avoid having to kill such large numbers, but can also increase the numbers that have to be killed due to a balance between inducing adverse effects in all animals as opposed to just some. Archiving (freezing down) rodent strains that are currently unwanted is a way of reducing the number of animals to be culled, as is accurately forecasting the number of animals to be used.

Recommendation: One way in which any poor welfare during euthanasia could be avoided is to not have to kill animals in the first place. Therefore, the production of animals should be carefully considered so that an avoidable surplus is not generated.

4.1.1. *Scientific reasons*

Occasionally, after considering all available methods, animals may have to be killed using methods that do not meet the animal welfare criteria set out for a humane method of killing for scientific reasons e.g. using some of the recognised methods may interfere with the scientific outcome. In a choice between two or more methods of humane killing, pilot studies may be carried out to determine the method that is most suitable for the scientific purpose and for the animals concerned. This may not always be the traditional method as new methods come along, or more information is gained on old methods questioning its humaneness, or its impact on the animal, its scientific validity and, therefore, its suitability. If animals are killed using less than ideal methods then that should be justified and taken into account when carrying out the harm (cost) benefit analysis. Some methods are listed in the report that cannot be considered humane, and are identified as such. For others, where there is a lack of information, that is addressed in future research.

Because the numbers of animal killed at any one time can range from one to several hundred, the method should be appropriate to dealing with both ends of the scale, again with the minimum distress to the animals as well as to the human operators.

Recommendation 1: In a choice between two or more methods of humane killing, the scientist should choose the most appropriate and humane but where this is not known pilot studies should be carried out.

As all methods have a margin of error it is important that death is confirmed, and if necessary ensured by the use of a method, such as exsanguination, freezing, or some physical insult that results in an irreversible destruction of the brain or central nervous system, or permanent cessation of the heart.

Recommendation 2: The death of an animal should be confirmed by a method that results in an irreversible destruction of the brain or permanent cessation of the heart.

4.2. Education, training and competence of those carrying out humane killing:

It is important that those carrying out such methods of killing are suitably trained and are deemed competent in that method (Council of Europe 1993). As nearly all methods require an element of restraint, it is equally important that they are competent in handling animals humanely.

The attitude of persons carrying out humane killing is important as over-sensitivity or a lack of care is more likely to result in poor welfare for the animals concerned. Killing animals in research establishments has been described as a kind of “initiation right” for animal care staff, and appropriate help and guidance should be available to guide young persons who are asked to do it (Arluke 1993, 1996). If senior staff members treat animals without sufficient respect, habits which lead to poor welfare may be formed in younger staff members. No-one should be coerced to kill animals, so scientists and others should be sensitive to the fact that those looking after animals did not enter this area of work to kill them; it is seen as an unavoidable, unpleasant aspect of animal care in research.

Recommendation 1: The humane killing of animals for *in vitro* and *ex vivo* research should be addressed so that persons carrying out such work are trained and competent.

Recommendation 2: A training plan should be drawn up, particularly for the use of physical methods that require a measure of manual skill, such as cervical dislocation or concussion, should incorporate a progression from the use of freshly killed animals, to anaesthetised animals, before going on to kill conscious animals. In that way there is less chance of poor welfare and poor scientific outcome due to poor technique.

4.3. Killing animals for their tissues:

Killing animals to retrieve tissues for *in vitro* work is outside the existing EU Directive (86/609/EEC), but such a use of animals is included in some countries (e.g. The Netherlands, Germany), and the number of animals used is counted giving an indication of the level of *in vitro* research by the scientific community. By including those animals killed for their tissues, the total annual number of animals used in research in those countries increased by 10 to 15%. Even though this use of animals is outside the Directive, there is EU and other national guidance on the ways by which animals should be humanely killed under laboratory conditions. Consequently, at present, research work involving killing animals by a recognised and approved method would permit, for example, researchers to kill 100 chimpanzees or dogs for a research purpose, without a licence, without oversight, and without any ethical or scientific approval. As death can be considered to be a lasting harm, it is debatable as to what level of licensing and scrutiny is required, and whether killing should be classified as a regulated procedure. In that case, animals killed for their tissues would receive the same level of care during euthanasia as an experimental animal and the staff would receive appropriate training and be certified competence as for any regulated procedure. Killing sick or injured stock animals could be exempted or encompassed.

Opinion: The humane killing of animals for *in vitro* and *ex vivo* research that, at present, is outside the Directive could cause public concern in regard to the species, the numbers and the competence of those carrying out the killing.

4.4. Gathering information

In order to know how often poor welfare occurs during euthanasia, we need to have quality control procedures and document when things go wrong and why, and what measures have been taken to stop it happening again. It is also important to know how often the method is used successfully so that an overall picture can be gained. This will then inform future risk assessments. At present this sort of information is not available, as it is in abattoirs in some countries.

Recommendation: Information should be collected on methods of euthanasia, e.g. their success rate in terms of an efficient and effective kill and the reasons for failure.

4.5. Methods of euthanasia

General comments applying to all methods:

The WG suggested that the recommended methods can be varied but only with a scientific justification and appropriate authority, i.e. the recommended methods represent the default position.

When pregnant animals are killed, the fetuses should be allowed to die *in utero* before being removed, unless they are required for scientific reasons, in which case they should be considered as neonates and killed by another method that is appropriate for the species and that causes a minimum of pain and distress.

4.5.1. *Electrical stunning*

Conclusions: Electrical methods, at present, are only used for farm animal species.

Equipment needs to be well maintained to function well.

The outcome depends on many variables including the equipment and the current delivered and also on the particular physical characteristics of the animal that might affect the effectiveness of the method.

Recommendations: Head-only electrical stunning and head-body killing can be recommended for the following adult species: rabbits, horses, donkeys and cross-bred equidae, pigs, goats, sheep, cattle and birds. Head-body stunning is recommended for fish. After electrical stunning an animal may recover with the consequence that it needs to be exsanguinated to be killed (or another method e.g. cooling down for fish). The unborn fetus will be killed by exsanguination or the cessation of blood supply due to heart failure of the pregnant dam.

Future Research: At present, there is considerable interest in the development in the electrical stunning of fish species. Since electrical techniques are easy to apply it may be worthwhile developing these methods for reptiles and amphibians.

The criteria used to determine a loss of consciousness in amphibia, reptiles, some fish species, and possibly some invertebrates are not well known and should be investigated.

4.5.2. Mechanical stunning methods

Conclusions: The penetrating captive bolt is an effective method of euthanasia for use in slaughterhouses and in research given adequate facilities in those species of animals in which the captive bolt has been specifically designed.

The equipment needs to be well maintained to function well.

Percussion stunning can be used for several species, however, there may be some doubts about effective stunning and killing in some animals. When correctly performed a concussive blow is very effective for smaller animals with ossified skulls, but it requires skill, confidence and practice (EFSA 2004).

Handling and restraint for concussive methods will cause some distress as the animal will be restrained in an unnatural position.

Recommendations: Concussive methods should not be used on animals with skulls that are not completely ossified or the sutures have not fused.

Future Research (probably depends on species): Water jet and air jet techniques and may be adaptable for many species.

4.5.3. Mechanical disruption of tissues (Neck dislocation, decapitation, maceration)

Conclusions:

1. Handling and restraint for neck dislocation and decapitation will cause some distress as the animal will be restrained in an unnatural position and will not be free to escape. Anaesthetising the animal first may reduce this distress.
2. After neck dislocation and decapitation electrical activity of the brain may persist for 13 s during which time animals may feel pain due to afferent stimuli from the trigeminal nerve. Cutting of the skin and tissues of the neck may cause some pain for a short period (less than one second).
3. After cervical dislocation, convulsions only occur when separation is made cranial to the fifth thoracic vertebra, while severance caudal to this location results in paralysis in conscious animals.
4. Mouse fetuses *in utero* are not killed within 20 min when the dam has been killed by cervical dislocation or decapitation. The heads of fetal rodents after decapitation may show signs of consciousness and this would be of welfare concern if the fetus had breathed (see Section 2.4).
5. After decapitation signs of consciousness may persist for some time e.g. 13 min in the heads of eels, and hours in reptiles.

6. If the macerator is overloaded animals may be not be humanely killed.
7. All these mechanical disruption techniques are aesthetically controversial. The interpretation of the electrical activity in the brain after neck dislocation and decapitation is controversial as to what feeling remains, and is still a matter of debate.
8. Anaesthetising animals before decapitation or cervical dislocation will minimise distress and any subsequent pain. This may be required in some cases of maceration where the animal may escape the blades.
9. Tissue damage to the CNS or induced neuronal discharge may affect neuropeptide levels and brain histology.
10. Severance of the spinal cord using a knife does not render the animal immediately unconscious and so it may suffer for some short time.

Recommendations:

1. When using these techniques, cervical dislocation and decapitation, the necessary handling and restraint can be stressful for the animal and anaesthetising them first will minimise distress and eliminate any subsequent pain.
2. A purpose built mechanical device with a sharp blade should be used for decapitation.
3. When pregnant females are killed the fetal forms should be allowed to die *in utero* before being removed, unless they are required for scientific reasons, in which case they should be killed by another method as quickly as possible.
4. Severance of the spinal cord using a knife should not be used.
5. For efficient and effective killing the macerator should not be overloaded.

Future Research: Since there are doubts that some species may not be immediately unconscious after neck-dislocation, alternative techniques should be developed.

4.5.4. Physical methods

Conclusions: Focal irradiation of the heads (brain) of restrained small animals with microwaves of 2450 MHz for 1s suggests a rapid loss of consciousness.

Focal heating of the brain by irradiation can only be applied by using a specially and constructed designed microwave oven specific for the species.

Hypothermia is not considered an acceptable method of euthanasia because it prolongs the period of consciousness and does not reduce the ability to feel pain.

Recommendations: Heating the brain focally with appropriately designed microwaves is accepted for use in adult rats and mice by trained operators and can be used for other animals such as guinea-pigs and hamsters when they are less than 300g.

Cooling down should not be used for any species.

Future Research: For many years, techniques using microwaves have been used for local damage of cells in cancer therapy. These techniques could be adapted to locally damage of brain tissue in a variety of species.

4.5.5. Gaseous methods

4.5.5.1. Exposure to carbon dioxide mixtures

Conclusions: CO₂ is aversive to all vertebrates used in research that have been tested. Some species find even low (10-20% by volume in air) concentrations aversive, regardless of any additions. It cannot be recommended as a sole method of humane killing for any species. CO₂ may be used as a secondary euthanasia procedure on unconscious animals.

Mouse fetuses *in utero* are not killed within 20 min even though the mother has been killed with CO₂, but it is possible to kill neonatal forms with CO₂.

Recommendation: Carbon dioxide should not be used as a sole agent in any euthanasia procedure unless the animal has first been rendered unconscious, i.e. it should be phased out as soon as possible. It is important that equally effective and non-aversive methods that are already partially developed, be developed further from a practical viewpoint, and that users are given time to change to those more humane gas mixtures.

It would be inappropriate to place a fully conscious animal in a known noxious gaseous environment from which it would be unable to escape.

Future Research: Research on euthanasia of animals should follow the guidelines set out by the International Association for the Study of Pain.

New methods of humane killing of animals using gas mixtures other than those containing CO₂ need urgently to be developed.

The time to onset of unconsciousness has usually been determined on the basis of behaviour (e.g. ataxia) but needs to be established more clearly using defined neurophysiological criteria.

An objective method of measuring breathlessness is needed to demonstrate and quantify breathlessness in laboratory animals (especially rodents), which would enable quantification of duration and severity of distress in animals exposed to any gas mixture.

4.5.5.2. Argon and Nitrogen as inert hypoxia inducing gases

Conclusions: It is suggested that the use of anoxia as a method of killing is humane for pigs and poultry, and probably rodents, although more practical experience is needed. Because of the high affinity for oxygen of haemoglobin in fetal and neonatal animals it may take longer than in mature animals of the same species to kill. However, no studies on time taken or welfare seem to have been carried out. More research is needed on nitrogen.

Recommendations: Research into hypoxic gas mixtures should be carried out as a matter of urgency, especially practical methods for small animals, such as rodents.

Future Research: Investigation is needed into the humaneness of killing with hypoxic and anoxic gas mixtures.

4.5.5.3. Nitrous oxide

Conclusions: Owing to human health and safety concern, nitrous oxide is not suitable for euthanasia.

Recommendations: (see Tables 1- 8)

Future Research: (probably species driven)

4.5.5.4. Carbon monoxide

Conclusions: Owing to human health and safety concern, carbon monoxide has a high risk for killing humans.

Recommendations: Under controlled conditions carbon monoxide can be used for dogs, cats and mink, however it is not recommended due to concerns for human health and safety, and also animal welfare.

4.5.5.5. Overdose of inhalation anaesthetic gases

Conclusion: Overdose of an established inhalational anaesthetic agent at a suitable concentration may cause minor distress in some species, but all such gases may be aversive at high concentrations. However, they have the advantage that restraint for administration is unnecessary.

Mouse fetuses *in utero* are not killed within 20 min even though the dam has been killed with an overdose, but neonatal forms (1-7 do) are killed.

Recommendation: Overdose of an inhalation anaesthetic agent should be considered as a humane way of killing animals providing some of the caveats relating to aversion and concentration are taken into consideration.

Future Research: Aversion testing may need to be carried out in some species for some agents (e.g. ferrets).

4.5.5.6. Overdose of injectable anaesthetic agents

Conclusion 1: Overdose of any anaesthetic agent may well be acceptable but all agents have some drawbacks in terms of irritancy and necessary restraint for administration. Suitable for mouse neonates (8-14 do) but not fetuses *in utero*.

Conclusion 2: In some member states some chemicals for euthanasia that cause a minimum of pain and distress may not be available.

Recommendation 1: Overdose of an injectable anaesthetic agent should be considered as a humane way of killing animals providing some of the caveats relating to aversion, irritancy and restraint are taken into consideration.

Recommendation 2: Member states should try to ensure that suitable chemicals for euthanasia are available.

4.5.5.7. Lethal injection of non-anaesthetising chemicals including: Neuromuscular blocking agents; Magnesium sulphate; Potassium chloride; Exposure to Hydrogen cyanide (HCN) gas; Ketamine; T-61

Conclusion: the administration of a non-anaesthetising chemical is potentially a major welfare problem.

Recommendation: Lethal injection of non-anaesthetising chemicals should only be administered in unconscious animals.

4.6. Humane killing of cephalopods, cyclostomes, decapods (if accepted)

Decapods include several kinds of crabs, lobsters and crayfish. Neither the number of crustaceans or cephalopods used in research is known and nor the methods of killing them are known. Although humane killing of crustaceans for food is not a statutory requirement in Europe, animal welfare organisations have provided some guidelines, for example, UFAW, RSPCA). In some countries, for example New Zealand, humane killing of some species of crustaceans is covered under the Animal Welfare Act 1999.

Recommendations:

The following methods cause a minimum of pain and distress:

- Chilling in air
- Chilling in ice/water slurry
- Immersion in a clove oil bath
- Electrical methods

The following methods are likely to cause pain and distress:

- Any procedure involving the separation of the abdomen (tailpiece) from the thorax (tailing) or removal of tissue, flesh or limbs while the crustacean is still alive and fully conscious (including when in a chilled state).
- Placing crustaceans in cold water and heating the water to boiling point.
- Placing live crustaceans into hot or boiling water.
- Placing live marine crustaceans in fresh water.
- Unfocussed microwaves to body as opposed to focal application to the head.

5. Tables with the recommended methods for the humane killing of animals in the laboratory.

Adapted and modified Tables from Close et al. (1996/1997)

The following tables have been taken from the previous EU Report on euthanasia, and form the basis for methods of killing laboratory animals that involve a minimum level of pain and distress. The data have been largely retained and only a few recommendations have been changed. (These tables in the scientific report are numbered as 7 to 14)

Table 1 - Characteristics of methods for euthanasia of fish

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
MS-222	++	++	++	++	++	5	Acceptable
Benzocaine	++	++	++	++	++	5	Acceptable
Etomidate	++	++	++	++	++	5	Acceptable
Metomidate	++	++	++	++	++	5	Acceptable
Electrical	++	+	+	+	++	4	Acceptable for some species
Maceration	++	++	++	++	+	4	Only for fish less than 2 cm in length
Quinaldine	++	++	++	+	++	4	Difficult to obtain in Europe
Concussion	++	+	+	++	-	3 *	Death to be confirmed Acceptable for use by experienced personnel
Sodium pentobarbitone	++	++	-	+	++	3	May be useful for large fish, intraperitoneal injection
Cervical dislocation	++	++	+	++	-	3	Not in large fish. To be followed by destruction of the brain
Halothane	+	+	++	++	++	2	Other methods preferable. Death to be confirmed

*Changed from Close et al. * was 4*

The following methods may only be used on unconscious fish: pithing, decapitation and exsanguinations

The following methods are not to be used for killing fish: removal from water, whole body crushing, hypothermia, hyperthermia, 2-phenoxyethanol, carbon dioxide, diethyl ether, secobarbital, amobarbital, urethane, chloral hydrate, tertiary amyl alcohol, tribromoethanol, chlorobutanol, methyl pentynol, pyridines, electrical stunning only for some species.

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 2 - Characteristics of methods for euthanasia of amphibians

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
MS-222	++	++	++	++	++	5	Acceptable
Benzocaine	++	++	++	++	++	5	Acceptable
Sodium pentobarbitone	+	++	-	+	+	4	Involves handling and intravenous or intraperitoneal injection
Concussion	++	++	+	++	- *	3 **	Acceptable for use by experienced personnel
T-61	+	++	-	+	+	3	Involves handling and intravenous injection
Microwave	++	++	-	+	++	3	Only for small amphibians. Not a routine procedure
Electrical stunning	+	+	+	-	-	2	To be followed immediately by destruction of the brain

Changed from Close et al. * was +, ** was 4

The following methods are only to be used on unconscious amphibians: pithing and decapitation

The following methods are not to be used for killing amphibians: hypothermia, hyperthermia, exsanguination, strangulation, carbon dioxide, diethyl ether, chloroform, volatile inhalational anaesthetics, chloral hydrate, ketamine hydrochloride, chlorbutanol, methylpentynol, 2-phenoxyethanol, tertiary amyl alcohol, tribromoethanol and urethane

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 3- Characteristics of methods for euthanasia of reptiles

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	++	+	++	5	Acceptable, but involves handling
Captive bolt	++	++	++	+	+	5	Acceptable for large reptiles
Shooting	++	++	++	-	+	4	Acceptable only in field conditions
Concussion	+	+	+	++	-	3**	Acceptable for use by experienced personnel To be followed by destruction of the brain

*Changed from Close et al. * was +; was 4*

The following methods are to be used on unconscious reptiles only: pithing and decapitation

The following methods are to be used on unconscious reptiles only: pithing and decapitation
 The following methods are not to be used for killing reptiles: spinal cord severance, hypothermia, hyperthermia, exsanguination, chloroform, MS-222, ether, halothane, methoxyflurane, isoflurane, enflurane, carbon dioxide, neuromuscular blocking agents, ketamine hydrochloride, chloral hydrate and procaine

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 4 - Characteristics of methods for euthanasia of birds

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
T-61	++	++	+	+	++	4	Requires expertise: acceptable for small birds only (<250 g)
Inert gases (Ar, N ₂)	++	++	++	++	+	4	Acceptable. But more research needed for nitrogen
Halothane, enflurane, isoflurane	++	++	++	+	++	4	Acceptable
Maceration	++	++	++	++	-	4	Acceptable for chicks up to 72 h
Cervical dislocation decapitation	++	++	-	++	-	3 **	Acceptable for small and young birds (<250 g) if followed by destruction of the brain
Microwave	++	++	-	++	+	3	To be used by experienced personnel only and specific equipment. Not a routine procedure
Concussion	++	++	-	++	-	3	Acceptable
Electrocution	++	++	+	-	-	3	Danger to operator. Use of special equipment Other methods Preferable
Carbon monoxide	+	+	++	-	-	1	Danger to operator

Changed from Close et al. * was +; was 4

The following methods may only be used on unconscious birds: decapitation, pithing, nitrogen, potassium chloride.

The following methods are not to be used for killing birds: neck crushing, decompression, exsanguination, carbon dioxide, nitrous oxide, diethyl ether, chloroform, cyclopropane, hydrogen cyanide gas, trichlorethylene, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, ketamine and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 5 - Characteristics of methods for euthanasia of rodents

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Halothane, enflurane, isoflurane	++	++	++	+	++	5	Acceptable
Sodium pentobarbitone	++	++	+	+	++	5	Acceptable
T-61	++	++	-	+	++	4	Only to be injected intravenously
*Inert gases (Ar)	++	+	++	+	+	4	Acceptable
Concussion	++	++	+	++	-	3	Other methods preferred; Acceptable for rodents under 1 kg. Death to be confirmed by cessation of circulation
Cervical dislocation	++	++	+	++	-	3	Other methods preferred; Acceptable for rodents under 150g. Death to be confirmed by cessation of circulation
Microwave	++	++	-	++	+	3	To be used by experienced personnel only. Not a routine procedure
Decapitation	+	+	+	++	-	2	Other methods preferred
*Carbon dioxide	+	++	++	+	++	1 if sole agent 5 if animal unconscious	To be used when animal unconscious i.e. overall rating then based on the method to induce unconsciousness
Carbon monoxide	+	+	+	-	++	1	Danger to operator
Rapid freezing	-	+	++	++	-	0	Not acceptable

* Changed from Close et al.

The following methods may only be used on unconscious rodents: rapid freezing, exsanguination, air embolism, potassium chloride and ethanol

The following methods are not to be used for killing rodents: carbon dioxide (when sole agent, but urgent research need for a replacement), hypothermia, decompression, strangulation, asphyxiation, drowning, nitrogen, nitrous oxide, cyclopropane, diethyl ether, chloroform, methoxyflurane, hydrogen cyanide gas, trichlorethylene, strychnine, nicotine, chloral hydrate, magnesium sulphate and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 6 - Characteristics of methods for euthanasia of rabbits

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	++	+	++	5	Acceptable
T-61	++	++	-	+	++	4	Acceptable. Intravenous injection only
Captive bolt	++	++	-	+	+	4	Requires skill. Death to be confirmed by another method
Cervical dislocation	++	++	-	++	-	3	Acceptable for rabbits under 1 kg. Sedation prior to dislocation. Death to be confirmed by cessation of circulation
Concussion	++	+	-	++	-	3	Expertise required. Death to be ensured by another method
Electrical stunning	++	+	++	-	+	3	Death to be confirmed by another method
Microwave	++	++	-	++	+	3	To be used by experienced personnel only on small rabbits. Not a routine procedure
Decapitation	+	+	+	-	-	2	Acceptable for rabbits under 1 kg if other methods not possible
Halothane, enflurane, isoflurane	++	++	++	+	-	2	Rabbits show signs of distress
Carbon monoxide	+	+	++	-	++	1	Danger to operator
Rapid freezing	+	+	++	++	+	1	Only in fetuses under 4 kg. Other methods preferred

Changed from Close et al.: CO2 deleted

The following methods are only to be used on unconscious rabbits: exsanguination, nitrogen, potassium chloride and air embolism.

The following methods are not to be used for killing rabbits: carbon dioxide, hypothermia, decompression, strangulation, asphyxiation, drowning, nitrous oxide, cyclopropane, diethyl ether, chloroform, trichlorethylene, hydrogen cyanide gas, methoxyflurane, chloral hydrate, strychnine, nicotine, magnesium sulphate, hydrocyanic acid, ketamine hydrochloride and neuro-muscular blocking agents.

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 7 - Characteristics of methods for euthanasia of dogs, cats, ferrets, foxes

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	-	+	++	5	Acceptable. Intravenous injection
T-61	++	++	-	+	+	4	Acceptable but only by slow intravenous Injectioninjection under sedation
Secobarbital/dibucaine	++	++	-	+	++	4	Acceptable. Intravenous injection
Halothane, isoflurane, enflurane	++	++	+	+	++	4	Acceptable
*Shooting with a free bullet with appropriate rifles and guns.	++	++	-	-	-	4 *	Acceptable only in field conditions by specialized marksmen when other methods not possible
Captive bolt	++	++	-	++	+	3	To be followed by exsanguination
Electrocution	++	++	-	-	-	3	Use only special equipment. To be followed by exsanguination
Concussion	++	++	+	++	-	2	Only to be used on neonates. To be followed by exsanguination

Changed from Close et al. * was 1

The following methods can be used for unconscious carnivores: exsanguination, neck dislocation and potassium chloride , in order to minimise pain and distress.

The following methods are not to be used for killing carnivores: decompression, decapitation, drowning, strangulation, asphyxiation, inert gases, nitrogen, air embolism, striking chest of cats, carbonmonoxide, carbon dioxide, methoxyflurane, nitrous oxide, trichlorethylene, hydrocyanic acid, diethyl ether, chloroform, hydrogen cyanide gas, cyclopropane, chloral hydrate, strychnine, nicotine, magnesium sulphate and neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, +acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

Table 8 - Characteristics of methods for euthanasia of large mammals

Agent	Rapidity	Efficacy	Ease of use	Operator safety	Aesthetic value	Overall rating (1-5)	Remarks
Sodium pentobarbitone	++	++	-	+	++	5	Acceptable by intravenous injection (all species including primates)
Quinalbarbitone/ Nupercaine	++	++	-	+	++	5	Effective for horses intravenously
Captive bolt	++	++	+	+	+	5	To be followed by exsanguination
Free bullet using e.g. appropriate ammunition, rifles and guns	++	++	+	-	+	4 *	Experienced marksman. May need a method to ensure death. In field conditions only.
T-61	++	++	-	+	++	4	Acceptable by intravenous injection
**Inert gases (Ar)	++	++	+	+	+	4	Acceptable for pigs only
Electrical stunning	++	++	+	-	-	4	Use only specialised equipment. To be followed immediately by exsanguination
Concussion	++	+	-	+	+	2	To be followed immediately by exsanguination
Halothane, isoflurane, enflurane	+	+	+	+	+	2	Recommended for lambs and kids

*Changed from Close et al. CO2 deleted, * was 5, ** introduced, CO2 deleted*

The following methods can be used only on unconscious large mammals: exsanguination, chloral hydrate and potassium chloride, in order to minimise pain and distress.

The following methods are not to be used for killing large mammals: carbon dioxide, carbon monoxide, methoxyflurane, trichlorethylene, strychnine, nicotine, magnesium sulphate, thiopentone sodium, ketamine hydrochloride, neuromuscular blocking agents

Rapidity: ++ very rapid, + rapid, - slow. **Efficacy:** ++ very effective, + effective, - not effective. **Ease of use:** ++ easy to use, + requires expertise, - requires specialist training. **Operator safety:** ++ no danger, + little danger, - dangerous. **Aesthetic value:** ++ good aesthetically, + acceptable for most people, - unacceptable for many people. **Rating:** 1-5 with 5 as highly recommended

6. DOCUMENTATION PROVIDED TO EFSA

Letter sent on the 23/07/2004 with ref. DG ENV. C JV/jm D (04) 430238, from Mr Jos Delbeke, from the Directorate-General Environment, Directorate C - Air and Chemicals

Supportive Documents

- The Commission sent, as background information, the EU reference on approved methods for euthanasia (Close *et al.*, 1996, 1997).

6.1. REFERENCES

All references are available in the scientific report.

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8. ACKNOWLEDGEMENTS

The working group drafted the scientific risk assessment, which was then reviewed and adopted by the AHAW Panel. The working group was chaired by David Morton on behalf of the AHAW Panel. The members of the working group were:

Questions 1&2 - Chairman Prof. Donald Broom: Dr Chris Sherwin, Prof. Neville Gregory and Dr Roddy Williamson

Question 3 – Chairman Dr Xavier Manteca: Prof Stefano Cinotti, Dr David Anderson and Prof. Timo Nevalainen

Question 4 - Chairman Prof David Morton: Dr Mohan Raj and Dr Bert Lambooj

The declarations of conflicts of interest of all participants in this working group will be available on internet, in the EFSA web site (<http://www.efsa.eu.int>)